

- ES
Conclude
- a) searching a sequence database using a suitable algorithm to identify an amino acid sequence which forms a helix-turn-helix structure or designing an amino acid sequence which forms a helix-turn-helix structure;
- b) substituting individual residues in said amino acid sequence to achieve a sequence having the same distribution of positively charged residues and cysteine residues as the distribution found in a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5;
- c) synthesizing chemically or expressing by recombinant DNA techniques in liquid culture an antimicrobial protein comprising said substituted amino acid sequence; and
- d) isolating said antimicrobial protein.

E9
Gulf

17. **(Amended three times)** The protein fragment of claim 1, wherein said protein fragment is a polypeptide comprising a cysteine and tyrosine or phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z (SEQ ID NOS: 34-36) wherein X and C are as defined in claim 1, and Z is tyrosine or phenylalanine.

E10
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18. **(Amended three times)** The protein fragment of claim 1, wherein said cysteine spacing comprises C-2X-C-3X-C-(10-12)X-C-3X-C-3X-C (SEQ ID NOS: 31-33) wherein X and C are as defined in claim 1.

E11
Gulf

30. **(Amended three times)** A composition comprising the protein fragment of claim 19 together with an agriculturally-acceptable carrier diluent or excipient.

E12
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34. **(Amended three times)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 11 for a period sufficient to reduce the number of said microbes.

E13
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43. **(Amended)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 30 for a period sufficient to reduce the number of said microbes.

44. **(Amended)** A method of reducing the number of fungi infesting a plant, the method comprising administering to said plant an effective amount of an antimicrobial protein for a period sufficient to reduce the number of said fungi, wherein:

said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue, and C is cysteine.

Q184
E-13
conclude

45. **(Amended)** A method of reducing the number of fungi infesting a plant, the method comprising administering to said plant an effective amount of a composition comprising an antimicrobial protein together with an agriculturally-acceptable carrier diluent or excipient for a period sufficient to reduce the number of said fungi, wherein:

said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue, and C is cysteine.

Please add the following claims:

E-14

46. **(New)** composition comprising the isolated or purified protein of claim 2 together with an agriculturally-acceptable carrier diluent or excipient.

Q184

47. **(New)** A composition comprising the isolated or purified protein of claim 3 together with an agriculturally-acceptable carrier diluent or excipient.

48. **(New)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the isolated or purified protein of claim 2 for a period sufficient to reduce the number of said microbes.

49. **(New)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the isolated or purified protein of claim 3 for a period sufficient to reduce the number of said microbes.

50. **(New)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 17 for a period sufficient to reduce the number of said microbes.

51. **(New)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 18 for a period sufficient to reduce the number of said microbes.

52. **(New)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 19 for a period sufficient to reduce the number of said microbes.

53. **(New)** A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 46 for a period sufficient to reduce the number of said microbes.

Appl. No. : 09/331,631
Filed : June 21, 1999

8/24/99
Conclude 54. (New) A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 47 for a period sufficient to reduce the number of said microbes.

REMARKS

The specification has been amended to correct minor informalities of spelling and an incorrect reference to a relevant embodiment of the invention. Support for amending the "tenth" embodiment on page 13 to the "twelfth" embodiment is found in the description of the twelfth embodiment beginning on page 5 at line 29 of the specification as filed. The amendments on page 26 of the specification have been made to correct typographical errors. Pages 4 and 10 have been amended to include the phrase "other than cysteine" to bring the description into conformity with the claims as sought and are discussed in further detail below.

Claims 32 and 33 have been canceled without prejudice and claims 3, 11, 13, 16-18, 30, 34 and 43-45 have been amended. Claims 32 and 33 have been cancelled for matters unrelated to patentability, specifically that they depend, either directly or indirectly, from previously cancelled claim 22 (see the response to Paper No. 12). Claim 3 has been amended to introduce Markush wording. Claim 11 has been amended to make the claim preamble consistent with antecedent claim 1. With regard to the amendment of part (a) of claim 16, this is based on, *inter alia*, the Example 8 disclosure where there is specific mention of the algorithms which can be used to identify helix-turn-helix structures. Claims 17, 18, 30 and 31 have been amended to address the Examiner's concerns in previous Office Actions in respect of other claims. Claims 17 and 18 have been further amended to bring the definitions of X and C into conformity with antecedent claim 1. Further amendments to claim 18 are supported in the filed specification, and sub-paragraph (iii) of claim 1 of the antecedent International Application, No. PCT/AU97/00874 (Publication No. WO 98/27805): see the description at page 10, lines 14 and 15. The substitution of "SEQ ID NOS: 31-33" for "SEQ ID NOS: 1-33" in claim 18 also brings the sequence identification into conformity with the actual sequence listings. Claims 13, 34 and 43-45 have been amended to positively recite the step of administering an effective amount of the subject compound or composition wherein the administration is for a period that is sufficient to reduce the number of microbes infesting the plant.